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## TECHNICAL MANUSCRIPT 28

# THE SEROLOGICAL ASSAY OF CULTURE FILTRATES FOR STAPHYLOCOCCUS ENTEROTOXIN

NOVEMBER 1962

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UNITED STATES ARMY  
BIOLOGICAL LABORATORIES  
FORT DETRICK

U.S. ARMY CHEMICAL-BIOLOGICAL-RADIOLOGICAL AGENCY  
U.S. ARMY BIOLOGICAL LABORATORIES  
Fort Detrick, Maryland

The work reported here was performed  
under Project 4B11-02-066, Special BW  
Operations, Research on Staphylococcus  
Enterotoxin. The expenditure order was  
2057.

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Project 4B11-02-066

November 1962

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#### ANIMAL RESEARCH

In conducting the research reported herein, the investigators adhered to "Principles of Laboratory Animal Care" as established by the National Society of Medical Research.

The agar diffusion technique of Oudin<sup>1</sup> has been used by Surgalla *et al*<sup>3</sup> to determine the relative concentrations of antigens in preparations of staphylococcal enterotoxin and to study its antigenic properties.<sup>3</sup> Bergdoll *et al*,<sup>5</sup> using the same technique, identified a precipitate band with the emesis-producing component of partially purified toxin and used this procedure as a guide in the purification of enterotoxin.

Since highly purified type B enterotoxin produced by the S6 strain of *Staphylococcus aureus* was available, estimations of enterotoxin by the gel diffusion and quantitative precipitin test were compared.

Purified enterotoxin prepared by Dr. E. J. Schantz, Fort Detrick, by a modification of the procedure of Bergdoll<sup>5</sup> was dissolved in the desired concentrations in 0.02 M phosphate-buffered saline, pH 7.4, containing 1:10,000 merthiolate. Antitoxin was obtained by the extensive immunization of rabbits with this preparation, emulsified in Freund's complete adjuvant. The initial dose of 37.6 micrograms was increased by fivefold amounts until a maximum dose of 3.0 milligrams was administered. Each rabbit received a total of 19.5 milligrams of toxin over a six-month period. All injections were given by the subcutaneous route; the time interval and dose were dependent upon the reaction of the individual rabbit. After each injection the animals responded with an increase in body temperature and a loss of weight. The pooled sera contained 0.68 milligram of antibody N per milliliter as determined by the quantitative precipitin test of Heidelberger and Randall<sup>6</sup>.

Standard curves for both the gel diffusion test and the precipitin test were prepared with the purified toxin and antitoxin. Agar diffusion tests were incubated at 30°C in a glass-walled water bath. The movement of the precipitate band was measured at about 24-hour intervals using a cathetometer. A single band of precipitate was formed in the interaction of these two reagents. Quantitative precipitin tests were incubated at 37°C for four hours plus 4°C for four days. Washed precipitates were analyzed for total N by the micro-kjeldahl technique. Tests of the supernatant serum obtained after centrifugation and removal of the precipitate never showed the simultaneous presence of both antigen and antibody.

The comparative tests were performed on a number of filtrates of *S. aureus* cultures grown in various media at 37°C for 18 to 24 hours. The cultures were centrifuged, and the clarified fluid was sterilized by filtration through a UF sintered glass filter. Undiluted filtrate or filtrate diluted 1:5 with the buffered saline was tested by the Oudin test. In the quantitative precipitin tests, 0.5-milliliter amounts of undiluted or diluted filtrate (1:5) were mixed with 0.5 milliliter of antitoxin.

Representative results are presented in Table I. Analysis of the data by the "t" test showed no significant difference between the results obtained with the two tests at the 95 per cent level. These tests indicated that excellent correlation existed between the results obtained by the gel diffusion and the quantitative precipitin tests.

TABLE I. COMPARISON OF STAPHYLOCOCCUS ENTEROTOXIN DETERMINATIONS BY THE GEL DIFFUSION AND THE QUANTITATIVE PRECIPITIN TESTS

SAMPLE	Dilution	ug enterotoxin per ml	
		Gel Diffusion	Precipitin
B		143.0	153.8
B	1:5	150.0	
A		57.5	90.8
A	1:5	48.0	
F		180.0	169.6
F	1:5	173.5	
C		19.0	24.8
1		24.5	27.6
2		48.0	46.8
3		37.0	33.6
5		44.0	41.6
7		31.5	35.6
OA		15.9	22.4

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